Article type: Original Article

Ancient origin and genetic segregation of canine circovirus infecting arctic foxes (*Vulpes lagopus*) in Svalbard and red foxes (*Vulpes vulpes*) in Northern Norway

Running title: Canine circovirus in arctic foxes and red foxes

Lorenza Urbani[1], Morten Tryland[2,3], Dorothee Ehrich[2], Eva Fuglei[3], Mara Battilani[1], Andrea Balboni[1]

[1]Department of Veterinary Medical Sciences, Alma Mater Studiorum–University of Bologna, Ozzano dell'Emilia, Bologna, Italy

[2]Arctic Infection Biology, Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Tromsø, Norway

[3]Norwegian Polar Institute, Fram Centre, Tromsø, Norway

e-mail addresses: Lorenza Urbani: lorenza.urbani2@unibo.it; Morten Tryland: morten.tryland@uit.no; Dorothee Ehrich: dorothee.ehrich@uit.no; Eva Fuglei: eva.fuglei@npolar.no; Andrea Balboni: a.balboni@unibo.it

Correspondence

Mara Battilani

Department of Veterinary Medical Sciences, Alma Mater Studiorum–University of Bologna, Ozzano dell'Emilia, Bologna, Italy.

Email: mara.battilani@unibo.it

Abstract

Canine circovirus (CanineCV) is a relatively new viral species, belonging to the family *Circoviridae*, whose pathogenic role is still uncertain. Since its first description in one domestic dog in 2011 from the USA, several reports have been documenting its distribution worldwide. Recently, CanineCV was also detected in wild animals such as wolves, foxes and badgers. In order to investigate the presence and the genetic characteristics of CanineCV in foxes of Arctic and Sub-Arctic regions, the presence of CanineCV DNA in internal organs (liver and spleen) of 51 arctic foxes (*Vulpes lagopus*) from Svalbard archipelago and 59 red foxes (*Vulpes vulpes*) from Northern Norway, sampled from 1996 to 2001 and from 2014 to 2018, respectively, was screened by real-time PCR. CanineCV was detected in 11/51 arctic foxes and in 10/59 red foxes, backdating the circulation of the virus at least to 1996 in the arctic fox population. The complete genome of 14 identified CanineCV was sequenced and analysed showing an identity higher than 80.8% with the reference strains available to date. According to the species demarcation threshold of 80% genome-wide nucleotide sequence identity for members of the family *Circoviridae* provided by International Committee on Taxonomy of Viruses (ICTV), all the CanineCV belong to a single species. Phylogenetic analysis revealed that all the CanineCV were subdivided into five main clusters with one including only CanineCV identified in foxes. Furthermore, CanineCV identified in arctic foxes and red foxes formed two distinct lineages. From these data we hypothesize that the viral transmission did not occur between the two species of foxes as a consequence of the lack of contact between the two hosts or that the virus acquired mutations in the time elapsed between the samplings.

Keywords

Arctic, arctic fox, Canine circovirus, Norway, red fox, Svalbard

1. Introduction

Circoviruses are non-enveloped viruses belonging to the genus *Circovirus* of the family *Circoviridae*, and contain a small, circular single-stranded DNA genome of approximately 2000 nucleotides (nt) in length (Breitbart et al., 2017). These viruses have an ambisense genome organisation with two major and inversely arranged open reading frames (ORFs) encoding for the replicase associated protein (Rep) and the capsid protein (Cap) (Kotsias et al., 2019). Canine circovirus (CanineCV) was first identified in serum from healthy domestic dogs in 2011 in the USA (Kapoor et al., 2012). Since then, the virus has been reported in dogs from several countries with a prevalence ranging from 3.8% to more than 30% (Anderson et al., 2017; Decaro et al., 2014; Dowgier et al., 2017; Gentil et al., 2017; Hsu et al., 2016; Li et al., 2013; Niu et al., 2020; Piewbang et al., 2018; Sun et al., 2019; Thaiwong et al., 2016; Zaccaria et al., 2016). Unlike the presence of CanineCV in dogs worldwide, little is known about the actual distribution of CanineCV in wild species. However, a few studies reported CanineCV in wolves, foxes and badgers with variable prevalence depending on the population studied (i.e. healthy versus unhealthy individuals), the diagnostic method used for virus detection, the type of biological sample tested and the geographic origin of samples (Bexton et al., 2015; De Arcangeli et al., 2020; Zaccaria et al., 2016). CanineCV infection is associated with a certain clinical state characterised by vasculitis, haemorrhage and enteritis in dogs (Anderson et al., 2017; Dowgier et al., 2017; Gentil et al., 2017; Kotsias et al., 2019; Li et al., 2013) and encephalitis in foxes (Bexton et al., 2015). Moreover, an association between CanineCV and canine parvovirus (CPV-2) infection in dogs has been reported (Anderson et al., 2017; Dowgier et al., 2017; Thaiwong et al., 2016; Zaccaria et al., 2016). To date, few data on the genetic variability of CanineCV infecting wild canids are available compared to the numerous genomic sequences of CanineCV obtained from domestic dogs that have allowed to propose different distinct phylogenetic clades for this virus (Niu et al., 2020; Sun et al., 2019). In addition, despite the recent discovery, the virus may have circulated among carnivorous populations much

longer and testing biological samples of domestic and wild canids taken before 2011 could help clarify this aspect.

The aim of this study was to investigate the presence and the genetic characteristics of CanineCV in arctic foxes (*Vulpes lagopus*) from the Svalbard archipelago, Norway, and red foxes (*Vulpes vulpes*) from Northern Norway (mainland) sampled from 1996 to 2018.

2. Materials and Methods

2.1. Study sites and sampling

Fifty-one arctic foxes from the High-Arctic Svalbard archipelago were included in this study (Figure 1A, TableS1). Arctic foxes, 25 females, 24 males and two animals with unidentified gender, were caught by local trappers using baited traps during the annual harvest at Spitsbergen, Svalbard, Norway (76–81°N, 15–25°E). Samples were collected during the trapping seasons (November 1st – March 15th) from the period 1996-2001. Carcasses were allocated to six sampling sites (Figure 1B) and frozen before they were delivered to Norwegian Polar Institute for laboratory measurements and sampling. Tissue samples were obtained from liver (n=47) and/or spleen (n=49).
Fifty-nine red foxes from the northernmost part of the Norwegian mainland, Finnmark County, were included in this study (Figure 1A, TableS1). Red foxes, 28 females and 31 males, were shot as part of legal culling programs in Finnmark County during the period 2014-2018. Red foxes were collected from two geographical regions: the Varanger Peninsula (70.4 °N, 29.5 °E), located at the border between the Sub-Arctic and the Low-Arctic zone, and the mountain region around the lake Iešjávri (69.6 °N, 24.4 °E; Figure 1C). Carcasses were frozen at -20 °C until laboratory measurements and sampling of liver (n=58) and/or spleen (n=58) at UiT The Arctic University of Norway.

Age was determined by counting the cementum annuli of a sectioned canine tooth (Grue & Jensen, 1976). Age ranged between one and 11 years (median 1) for arctic foxes and between one and eight years (median 2) for red foxes.

2.2. Detection of the CanineCV DNA

DNA extraction from tissue samples of 25-50 mg was carried out by using the Maxwell 16 Tissue DNA Purification Kit (Promega) and the automatic extractor Maxwell 16 System (Promega) according to the manufacturer's instructions. Extracted DNA was stored at -20 °C until use. The presence of CanineCV DNA was screened by using a SYBR Green Real-time polymerase chain reaction (qPCR) targeting a highly conserved fragment of 132 nt in the intergenic region (IR) between the 3′ ends of the two major ORFs, with the primers CanineCV 909-931 qPCR-For (5′-CTGAAAGATAAAGGCCTCTCGCT-3′) and CanineCV 1020-1040 qPCR-Rev (5′-AGGGGGGTGAACAGGTAAACG-3′) (De Arcangeli et al. 2020). The reaction was performed using the PowerUp SYBR Green master mix (Thermo Fisher Scientific) in a total volume of 20 µL containing 0.3 µM of each primer, 2X Master Mix and 2 µL DNA in the StepOnePlus qPCR system (Thermo Fisher Scientific). The thermal cycling consisted of 95 °C for 5 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. Melting experiment for the evaluation of the specificity of the reaction was performed after the last extension step by a continuous increment from 55 °C to 99 °C and specific melting temperature ranged from 93.2 °C to 93.6 °C. CanineCV DNA copies number determination was carried out by absolute quantification using the standard curve method. Serial 10-fold dilutions of a plasmid (pCR4 plasmid; Life Technologies) containing one copy of the CanineCV target sequence were used as external standards for the construction of the assay standard curve by plotting the plasmid copy number against the corresponding threshold cycle values. The limit of detection (LOD) of the reaction was determined based on the highest dilution of recombinant plasmid possible to amplify with good reproducibility and was found to be five copies/µL. The DNA samples and standards were repeated within each run in duplicate. A no template control, consisting of ultrapure water, underwent analysis simultaneously. Samples showing an exponential increase in the fluorescence curve, a target DNA amount greater than or equal to the LOD and a specific melting peak in both replicates were considered positive.

2.3. Amplification and sequencing of the CanineCV complete genome

The complete genome of CanineCV was amplified from positive samples by integrating rolling circle amplification (RCA) and end-point PCR methods (De Arcangeli et al., 2020). The RCA was performed to increase the amount of circular DNA using the TempliPhi 100 amplification kit (GE Healthcare) following the manufacturer's instructions. Briefly, after an incubation at 95 °C for 3 min of a mix containing 10 µL of Sample buffer and 5 µL of sample DNA, 10 µL of a second mix containing TempliPhi Reaction buffer, TempliPhi Enzyme Mix and dNTPs were added to each samples, incubated at 30 °C for 16 h and inactivated at 65 °C for 10 min. A positive control DNA supplied by the manufacturer and a no template control, consisting of ultrapure water, were added. Subsequently, two overlapping regions of the viral genome were amplified by end-point PCR using two sets of primers, respectively: CanineCV_1.020-1.040_For (5′-CGTTTACCTGTTCACCCCCCT-3′) – CanineCV_909-931_Rev (5′-AGCGAGAGGCCTTTATCTTTCAG-3′) targeting a region of 1932 nt and CanineCV_3′-3′_For (5′-ATGGTGGGATGGCTACGATG-3′) – CanineCV_3′-3′_Rev (5′-CAAGGAAGAGGGAATGCTACAAG-3′) targeting a region of 936 nt (De Arcangeli et al., 2020). A proofreading DNA polymerase (Phusion Hot Start II High-Fidelity DNA Polymerase, Thermo Fisher Scientific) was used. The reactions were performed in a total volume of 50 µL containing 0.5 µM of each primer, 5X HF buffer, 2.5mM dNTP, 2U/µL Phusion Hot Start II DNA Polymerase and 5 µL of RCA product. The thermal cycling consisted of an initial denaturation at 98 °C for 30 s followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 65.3 °C or 63.7 °C (for the first and the second set of primers mentioned above, respectively) for 30 s and elongation at 72 °C for 1 min, followed by a final elongation step at 72°C for 10 min. A DNA extract of a CanineCV positive sample was used as positive control (09-10F/2011, GenBank ID: MH454599, De Arcangeli et al., 2020). A no template control, consisting of ultrapure water, underwent analysis simultaneously. PCR products (5 µL) were separated by electrophoresis in a 1.0% agarose gel in TAE buffer and

153 visualised by UV light after staining with SYBR Safe DNA Gel Stain (Bio-Rad Laboratories).
154 Amplicons of the expected size were considered positive, purified using the QIAquick PCR
155 Purification Kit (QIAGEN) according to the manufacturer's instructions and directly sequenced by
156 Sanger method (BioFab Research) using both forward and reverse primers. For foxes that showed
157 CanineCV specific qPCR products in more than one organ, the amplicon that showed the highest
158 amount of target DNA and no non-specific products, was sequenced. The complete CanineCV
159 genome sequences obtained in this study are openly available in INSDC database
160 (http://www.insdc.org/; ID: MT180077-MT180090).
161
162 ***2.4. Sequence analysis***
163 Complete viral genomes were assembled, aligned with 96 reference sequences of CanineCV
164 from GenBank (Table S2) using the ClustalW method implemented in BioEdit 7.2.5 and translated
165 into amino acid sequences. Phylogeny was carried out on complete genome nucleotide sequences
166 and concatenated Rep and Cap amino acid sequences using the software MEGA X version 10.1.7
167 (Kumar et al., 2018). Phylogenetic trees were constructed using Maximum Likelihood method and
168 the best-fit model of nucleotide substitution was determined for both alignments using the Find Best
169 DNA/Protein Model function implemented in MEGA X. The General Time Reversible (GTR)
170 model with gamma distribution and invariable sites resulted optimal for the nucleotide alignment,
171 while the Jones-Taylor-Thornton (JTT) model with gamma distribution and invariable sites resulted
172 optimal for the amino acid alignment. The robustness of individual nodes on the phylogeny was
173 estimated using 1000 bootstrap replicates and bootstrap values >70 were indicated at the
174 corresponding node.
175
176 **3. Results**
177 ***3.1. Detection of CanineCV DNA***

Eleven out of 51 arctic foxes and 10/59 red foxes tested positive for CanineCV DNA. CanineCV specific amplicons were generated from both sampled organs (liver and spleen) from nine of the 11 positive arctic foxes and five of the 10 positive red foxes. From the remaining foxes, such amplicons were generated from the spleen, except for one arctic fox which tested positive on the liver sample only. The overall median quantity of CanineCV DNA copies per microliter of spleen and liver DNA extract was 2.3×10^4 (range 7.7×10^1 - 1.7×10^6) in arctic foxes and 5.1×10^2 (range 6.7×10^1 and 3.3×10^5) in red foxes. For each fox species, the median amount of viral DNA detected in the extracts of the two tissues was of the same order of magnitude. Signalment data and positivity to CanineCV DNA of the foxes included in this study are reported in Table 1 (summary) and in Table S1 (detailed per animal).

3.2. Full-genome characterization

The complete CanineCV genome sequence was obtained from 8 arctic foxes and 6 red foxes. The genome of the 14 CanineCV sequenced in this study was 2063 nt in length. Genome characteristics include two major ORFs, ORF1 (912 nt) on the virion strand and ORF2 (813 nt) on the complementary strand of the replicative form, encoding for the Rep (303 amino acids) and the Cap (270 amino acids), respectively. Two intergenic noncoding regions of 135 and 203 nt in length, respectively, were located between the start and stop codons of the two major ORFs. The 5' IR, located between the start codons, contains a characteristic stem-loop structure with a conserved 9 nt motif (TAGTATTAC) for initiation of the rolling cycle replication.

Nucleotide alignment between the complete genomic sequences of CanineCV obtained in this study and reference strains showed an overall nucleotide identity ranging from 80.8 to 100%. All the sequences obtained in this study were different from each other and distinguishable in two groups: one composed by CanineCV identified in arctic foxes and one composed by CanineCV identified in red foxes, since they had a greater nucleotide identity within the groups (97.7-99.8% and 94.3-99.3%, respectively) rather than between the two groups (91.9-93.9%)..

The two unrooted phylogenetic trees constructed with complete genome nucleotide sequences (Figure 2) and with concatenated amino acid sequences of predicted Rep and Cap protein (Figure 3) showed a well distinguishable clustering of the CanineCV nucleotide sequences into five groups. Group 1 included CanineCV identified in dogs, wolves and a badger from Europe, USA and China. The groups 2, 3 and 4 includes CanineCV identified in dogs from Asia, with the exception of virus 09-10F/2011, which was identified in a red fox in Italy in 2011 (GenBank ID: MH454599; De Arcangeli et al., 2020) and allocated to group 4. Group 5 was only composed by CanineCV identified in foxes, including the sequences obtained in this study, and showed a well distinct origin in particular in the amino acidic tree (Figure 3). In this latter group, two lineages composed by CanineCV identified in arctic foxes and in red foxes, respectively, were evidenced and further distinguishable for geographic origin and years of sampling. Only in the nucleotide tree the CanineCV 55590, identified in a red fox in Croatia in 2014 (GenBank ID: KP941114, Lojkić et al., 2016), formed a distinct lineage within group 5 (Figure 2). The CanineCV obtained from a dog in the USA in 2011 (GenBank ID: KC241983, Li et al., 2013) did not cluster in any of the five groups, neither when comparing the nucleotide sequences nor the amino acid sequences. The nucleotide identity values calculated for the CanineCV sequences belonging to the same cluster and between different clusters identified in the phylogenetic analysis are reported in Table 2.

4. Discussion

In this study, we investigated the spread of CanineCV infection in arctic foxes from the Svalbard archipelago, Norway, and red foxes from mainland Northern Norway (1996 to 2018), and analysed the complete genome of the identified viruses. The CanineCV DNA was detected in liver and/or spleen samples of 11/51 arctic foxes and in 10/59 red foxes. A median quantity of viral DNA greater in tissues of arctic foxes than in red foxes was detected. Nevertheless, the impossibility of correlate the number of CanineCV DNA copies detected per microliter of extract to the exact amount of extracted tissue and the extreme variability of the time elapsed from the death

of the foxes to the sampling, with variable degradation of the tissues, do not allow to draw conclusions. Further studies, as a controlled inoculation study, are needed to better evaluate this aspect. The detection of CanineCV DNA in arctic foxes sampled between 1996 and 2001 represent the first report for this wild species and suggest that the virus circulated in arctic foxes at least fifteen years before its first discovery in domestic dog sera in the USA (Kapoor et al., 2012). Accordingly, we suggest that wild carnivores may have harboured an ancestor of CanineCV, as speculated by numerous studies on CPV-2, a similar small single-stranded DNA virus, in which wild hosts played a key role in the emergence of infection in dogs (Allison et al., 2012, 2013; Frölich et al., 2005; Truyen et al., 1995). The number of red foxes tested positive for CanineCV DNA in this study (10/59) is lower than reported in United Kingdom (7/15 in healthy red foxes and 13/17 in red foxes with neurologic signs, Bexton et al. 2015) and higher than reported in Italy by De Arcangeli at al. (1/32, 2020) and Zaccaria et al. (0/24, 2016). These discrepancies could be due to a different viral spread in the geographic areas investigated, but also to the health status of the sampled animals or the type of biological samples tested in the different studies. Indeed, serum, faeces and several internal organs (spleen, tonsil, lymph nodes, liver, intestine, lung, kidney and brain) samples have been tested in foxes for the molecular detection of CanineCV infection (Bexton et al., 2015; De Arcangeli et al., 2020; Zaccaria et al., 2016), but the limited knowledge available to date on the pathogenesis and clinical manifestations of the virus in wild hosts make it difficult to choose the optimal organ sample for viral DNA detection.

From the complete genome alignment carried out in this study, all the CanineCV sequences identified in dogs, foxes, wolves and badgers showed a nucleotide identity ≥80.8 %. According to the species demarcation threshold of 80% genome-wide nucleotide sequence identity for members of the family *Circoviridae* provided by Breitbart et al. (2017) and Rosario et al. (2017), this result confirms the existence of a unique circovirus species (nominally canine circovirus), infecting dogs, foxes, wolves and badgers and including the viruses detected in this study.

Phylogeny provided evidence of cluster formation of the CanineCV sequences into five groups, except for CanineCV UCD3-478 which could represent an intermediate sequence between groups 4 and 5 or the only virus to date sequenced of a further sixth group. Four of the five groups (named 1 to 4) correspond to the genotypes proposed by Niu et al. (2020). A fifth group, the number 5, composed only by CanineCV identified in foxes, included the viruses detected in this study, confirming a general clustering on geographic and/or host basis (Niu et al., 2020). In particular, the existence of a group of viruses infecting foxes, phylogenetically distinguishable from those infecting dogs, wolves and badgers, was evidenced as previously suggested by Zaccaria et al. (2016). However, not all the CanineCV detected in foxes clustered together in group 5, indeed the CanineCV 09-10F/2011 identified in a red fox clustered in group 4 with other viruses identified in dogs. Therefore, the CanineCV sequences do not group strictly on the basis of the host species in which they were identified. Further studies would be needed to investigate the susceptibility of domestic and wild carnivores to genetically different CanineCV with experimental or in vitro infections. Within group 5, the CanineCV identified in both arctic foxes and red foxes formed two distinct lineages. This genetic distance could be explained by the geographical segregation of the two fox species populations investigated in this study. Indeed, red foxes from the Norwegian mainland do not get in direct contact with arctic foxes in the archipelago of Svalbard (direct line distance is 835 km over the Barents Sea, that is not covered by sea ice), making a viral transmission between the two hosts at those locations impossible. Possible routes of transmission were also absent between arctic foxes in Svalbard and red foxes from Great Britain and Croatia in which the other CanineCV included in this group were identified. Nevertheless, indirect viral transmission between the two species populations could be possible through migration of arctic foxes to Svalbard from other arctic regions in which red foxes are present by using the sea ice as platform (Geffen et al., 2007; Henttonen et al., 2001; Mørk et al., 2011; Norén et al. 2011) or through domestic dogs (including sled dogs) brought from the Norwegian mainland to the Svalbard archipelago. Another hypothesis that may explain the sequence diversity that emerged between the CanineCV identified

in arctic foxes and red foxes, respectively, is the acquisition of mutations during the time elapsed between the sampling of the two species.

5. Conclusions

The present study reports the detection of CanineCV DNA in arctic foxes from the Svalbard archipelago, Norway, since 1996, fifteen years before the first report in domestic dogs in USA. Sequences analysis showed that CanineCV identified in arctic foxes from Svalbard and red foxes from mainland Norway represent two distinct lineages in a well distinct phylogenetic group, separated from other CanineCV. Currently, very little is known about the pathogenic role of CanineCV in wild carnivores and the possible transmission of the virus between wild animals and domestic dogs. Continuous epidemiological surveillance is therefore needed to understand the importance and evolution of CanineCV in wild animals and to characterise the potential pathogenic impact CanineCV may have on dogs and on wild carnivore species and populations.

Acknowledgments

We thank all the trappers and hunters that provided the fox samples. Funding for sampling the fox tissues were provided from the Norwegian Polar Institute and financed by the Norwegian Environment Agency to Climate – ecological Observatory for Arctic Tundra (COAT). We also acknowledge Dr. Stefano De Arcangeli for help with the tissue samples and DNA extraction, Dr. Chiara Scarano for help with molecular analyses and we thank Anders Skoglund, Norwegian Polar Institute, for graphical design of maps.

Authors' contributions

LU wrote the original manuscript draft. LU and AB performed tests acquiring the sequence data. MT, DE, EF collected samples and data. LU, MT and AB analysed the data. MT, MB and AB

conceived and designed the study and critically revised the manuscript. All authors read, commented on and approved the final drafts of the manuscript.

Ethical Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as sample collection was obtained from dead animals and no life animal handling was performed in order to conduct the present study.

Conflict of Interest Statement

The authors have no financial or personal interests that could influence or bias the content of this article. The authors declare that they have no competing interests. All authors have seen and approved the manuscript.

Data Availability Statement

The data that support the findings of this study are openly available in INSDC (http://www.insdc.org/; ID: MT180077-MT180090).

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

References

Allison, A.B., Harbison, C.E., Pagan, I., Stucker, K.M., Kaelber, J.T., Brown, J.D., … Parrish, C.R. (2012). Role of multiple hosts in the cross-species transmission and emergence of a

pandemic parvovirus. *Journal of Virology*, *86*, 865-872. https://doi.org/10.1128/JVI.06187-11

Allison, A.B., Kohler, D.J., Fox, K.A., Brown, J.D., Gerhold, R.W., Shearn-Bochsler, V.I., … Holmes, E.C. (2013). Frequent cross-species transmission of parvoviruses among diverse carnivore hosts. *Journal of Virology*, *87*, 2342-2347. https://doi.org/10.1128/JVI.02428-12

Anderson, A., Hartmann, K., Leutenegger, C.M., Proksch, A.L., Mueller, R.S., Unterer, S. (2017). Role of canine circovirus in dogs with acute haemorrhagic diarrhoea. *Veterinary Record*, *180*, 542. https://doi.org/10.1136/vr.103926

Bexton, S., Wiersma, L.C., Getu, S., van Run, P.R., Verjans, G.M., Schipper, D., … Smits, S.L. (2015). Detection of circovirus in foxes with meningoencephalitis, United Kingdom, 2009-2013. *Emerging Infectious Diseases*, *21*, 1205-1208. https://doi.org/10.3201/eid2107.150228

Breitbart, M., Delwart, E., Rosario, K., Segalés, J., Varsani, A. (2017). ICTV virus taxonomy profile: Circoviridae. *Journal of General Virology*, *98*, 1997-1998. https://doi.org/10.1099/jgv.0.000871

De Arcangeli, S., Balboni, A., Kaehler, E., Urbani, L., Verin, R., Battilani, M. (2020). Genomic characterization of canine circovirus detected in red foxes (*Vulpes vulpes*) from Italy using a new Real-Time PCR assay. *Journal of Wildlife Diseases*, *56*, 239-242. https://doi.org/10.7589/2018-11-270

Decaro, N., Martella, V., Desario, C., Lanave, G., Circella, E., Cavalli, A., … Buonavoglia, C. (2014). Genomic characterization of a circovirus associated with fatal hemorrhagic enteritis in dog, Italy. *PLoS One*, *9*, e105909. https://doi.org/10.1371/journal.pone.0105909

Dowgier, G., Lorusso, E., Decaro, N., Desario, C., Mari, V., Lucente, M.S., … Elia, G. (2017). A molecular survey for selected viral enteropathogens revealed a limited role of canine circovirus in the development of canine acute gastroenteritis. *Veterinary Microbiology*, *204*, 54-58. https://doi.org/10.1016/j.vetmic.2017.04.007

Frölich, K., Streich, W.J., Fickel, J., Jung, S., Truyen, U., Hentschke, J., … Latz, N. (2005). Epizootiologic investigations of parvovirus infections in free-ranging carnivores from Germany. *Journal of Wildlife Diseases, 41*, 231-235. https://doi.org/10.7589/0090-3558-41.1.231

Geffen, E., Waidyaratne, S., Dalén, L., Angerbjörn, A., Vila, C., Hersteinsson, P., … Wayne, R.K. (2007). Sea ice occurrence predicts genetic isolation in the Arctic fox. *Molecular Ecology*, *16*, 4241-4255. https://doi.org/10.1111/j.1365-294X.2007.03507.x

Gentil, M., Gruber, A.D., Müller, E. (2017). [Prevalence of dog circovirus in healthy and diarrhoeic dogs]. *Tierarztliche Praxis Ausgabe K Kleintiere Heimtiere*, *45*, 89-94. https://doi.org/10.15654/TPK-160701

Grue, H., & Jensen, B. (1976). Annual cementum structures in canine teeth in Arctic foxes (*Alopex lagopus L.*) from Greenland and Denmark. *Danish review of game biology*, *10*(3), 1-12.

Henttonen, H., Fuglei, E., Gower, C.N., Haukisalmi, V., Ims, R.A., Niemimaa, J., Yoccoz, N.G. (2001). *Echinococcus multilocularis* on Svalbard: introduction of an intermediate host has enabled the local life-cycle. *Parasitology*, *123*, 547-552. https://doi.org/10.1017/S0031182001008800

Hsu, H.S., Lin, T.H., Wu, H.Y., Lin, L.S., Chung, C.S., Chiou, M.T., Lin, C.N. (2016). High detection rate of dog circovirus in diarrheal dogs. *BMC Veterinary Research, 12*, 116. https://doi.org/10.1186/s12917-016-0722-8

Kapoor, A., Dubovi, E.J., Henriquez-Rivera, J.A., Lipkin, W.I. (2012). Complete genome sequence of the first canine circovirus. *Journal of Virology, 86*, 7018. https://doi.org/10.1128/JVI.00791-12

Kotsias, F., Bucafusco, D., Nuñez, D.A., Lago Borisovsky, L.A., Rodriguez, M., Bratanich, A.C. (2019). Genomic characterization of canine circovirus associated with fatal disease in dogs in South America. *PLoS One, 14*, e0218735. https://doi.org/10.1371/journal.pone.0218735

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547-1549. https://doi.org/10.1093/molbev/msy096

Li, L., McGraw, S., Zhu, K., Leutenegger, C.M., Marks, S.L., Kubiski, S., … Pesavento, P.A. (2013). Circovirus in tissues of dogs with vasculitis and hemorrhage. *Emerging Infectious Diseases*, *19*, 534-541. https://doi.org/10.3201/eid1904.121390

Lojkić, I., Biđin, M., Prpić, J., Šimić, I., Krešić, N., Bedeković, T. (2016). Faecal virome of red foxes from peri-urban areas. *Comparative Immunology, Microbiology and Infectious Diseases*, *45*, 10-15. https://doi.org/10.1016/j.cimid.2016.01.005

Mørk, T., Bohlin, J., Fuglei, E., Åsbakk, K., Tryland, M. (2011). Rabies in the arctic fox population, Svalbard, Norway. *Journal of Wildlife Diseases*, *47*, 945-957. https://doi.org/10.7589/0090-3558-47.4.945

Niu, L., Wang, Z., Zhao, L., Wang, Y., Cui, X., Shi, Y., … Ge, J. (2020). Detection and molecular characterization of canine circovirus circulating in northeastern China during 2014-2016. *Archives of Virology*, *165*, 137-143. https://doi.org/10.1007/s00705-019-04433-4

Norén, K., Carmichael, L., Fuglei, E., Eide, N.E., Hersteinsson, P., Angerbjörn, A. (2011). Pulses of movement across the sea ice: population connectivity and temporal genetic structure in the arctic fox. *Oecologia*, *166*, 973-984. https://doi.org/10.1007/s00442-011-1939-7

Piewbang, C., Jo, W.K., Puff, C., van der Vries, E., Kesdangsakonwut, S., Rungsipipat, A., … Osterhaus, A.D.M.E. (2018). Novel canine circovirus strains from Thailand: Evidence for genetic recombination. *Scientific Reports*, *8*, 7524. https://doi.org/10.1038/s41598-018-25936-1

Rosario, K., Breitbart, M., Harrach, B., Segalés, J., Delwart, E., Biagini, P., Varsani, A. (2017). Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. *Archives of Virology*, *162*, 1447-1463. https://doi.org/10.1007/s00705-017-3247-y

Sun, W., Zhang, H., Zheng, M., Cao, H., Lu, H., Zhao, G., … Jin, N. (2019). The detection of canine circovirus in Guangxi, China. *Virus Research*, *259*, 85-89. https://doi.org/10.1016/j.virusres.2018.10.021

Thaiwong, T., Wise, A.G., Maes, R.K., Mullaney, T., Kiupel, M. (2016). Canine circovirus 1 (CaCV-1) and canine parvovirus 2 (CPV-2): recurrent dual infections in a papillon breeding colony. *Veterinary Pathology*, *53*, 1204-1209. https://doi.org/10.1177/0300985816646430

Truyen, U., Gruenberg, A., Chang, S.F., Obermaier, B., Veijalainen, P., Parrish, C.R. (1995). Evolution of the feline-subgroup parvoviruses and the control of canine host range in vivo. *Journal of Virology*, *69*, 4702-4710. https://doi.org/10.1128/JVI.69.8.4702-4710.1995

Zaccaria, G., Malatesta, D., Scipioni, G., Di Felice, E., Campolo, M., Casaccia, C., … Lorusso, A. (2016). Circovirus in domestic and wild carnivores: An important opportunistic agent? *Virology*, *490*, 69-74. https://doi.org/10.1016/j.virol.2016.01.007

Table 1 Signalment data and positivity to canine circovirus DNA of the arctic foxes (*Vulpes lagopus*) and red foxes (*Vulpes vulpes*) included in this study

	Arctic foxes			Red foxes		
Variables	**Positive**	**Negative**	**Total**	**Positive**	**Negative**	**Total**
Number of foxes	11	40	51	10	49	59
Sex						
Male	7	17	24	6	25	31
Female	4	21	25	4	24	28
NA	-	2	2	-	-	-
Geographical origin						
Svalbard archipelago (Norway)						
Adventdalen	-	1	1	-	-	-
Austfjordnes	3	8	11	-	-	-
Bjonehamna	1	-	1	-	-	-
Bjørndalen	-	1	1	-	-	-
Coles Bay	1	-	1	-	-	-
Colesdalen/Grumant	3	5	8	-	-	-
Flowerdalen	-	1	1	-	-	-
Fredheim	-	1	1	-	-	-
Janssonhaugen	1	2	3	-	-	-
Kapp Wijk	1	11	12	-	-	-
Koslodalen	-	1	1	-	-	-
Kapp Murdoch	-	1	1	-	-	-
Nordenskiöld Land	-	5	5	-	-	-
Reindalen	-	1	1	-	-	-
Templet	1	-	1	-	-	-
NA	-	2	2	-	-	-
Finnmark County (Norway)						
Iešjávri	-	-	-	7	19	26
Varanger Peninsula	-	-	-	3	30	33
Year of sampling						
1996	1	3	4	-	-	-
1997	6	23	29	-	-	-
1998	1	2	3	-	-	-
1999	3	11	14	-	-	-
2001	-	1	1	-	-	-
2014	-	-	-	2	13	15
2015	-	-	-	2	10	12
2016	-	-	-	1	9	10
2017	-	-	-	3	10	13
2018	-	-	-	2	7	9
Age (years) [†]	1 [1-3]	1 [1-11]	1 [1-11]	2 [1-3]	2 [1-8]	2 [1-8]

[††]Data are reported as median [minimum value – maximum value].

Note: NA = not available.

Table 2 Nucleotide identities (%) among complete genome sequences (2063-2064 nt) belonging to the clusters identified in the phylogenetic analysis. The sequences used for alignment are shown in the Table S2

	Group 1	Group 2	Group 3	Group 4	Group 5	AF	RF	UCD3-478
Group 1	93.5-100	83.8-87.3	88.7-90.4	85.9-92.6	82.5-84.9	82.6-84.7	82.5-84.9	84.6-86.8
Group 2	83.8-87.3	89.1-99.9	85.1-90.1	84.8-90.6	80.8-83.6	81.2-83	80.8-83.6	81.9-84.6
Group 3	88.7-90.4	85.1-90.1	96.4-99.8	85.9-89.8	82.1-83.8	82.4-83.4	82.1-83.8	84.3-84.5
Group 4	85.9-92.6	84.8-90.6	85.9-89.8	92.4-99.9	84.4-86.6	84.4-85.8	84.6-86.6	88.8-90.9
Group 5	82.5-84.9	80.8-83.6	82.1-83.8	84.4-86.6	91.9-99.8	-	-	88.8-89.9
AF	82.6-84.7	81.2-83	82.4-83.4	84.4-85.8	-	97.7-99.8	91.9-93.9	88.9-89.1
RF	82.5-84.9	80.8-83.6	82.1-83.8	84.6-86.6	-	91.9-93.9	94.3-99.3	88.9-89.9
UCD3-478	84.6-86.8	81.9-84.6	84.3-84.5	88.8-90.9	88.8-89.9	88.9-89.1	88.9-89.9	100

Data are expressed as range from the minimum identity value to the maximum identity value. Nucleotide identities among sequences of CanineCV identified in this study in arctic foxes and red foxes and sequences of CanineCV within group 5 have not been reported because they are themselves included in group 5.

Note: AF = CanineCV identified in this study in arctic foxes; RF = CanineCV identified in this study in red foxes.

Fig. 1. Sampling locations. (A) Arctic foxes (*Vulpes lagopus*) were sampled on Spitsbergen, Svalbard, and red foxes (*Vulpes vulpes*) in Finnmark County, mainland Norway. (B) Arctic foxes were sampled from six areas on Spitsbergen, Svalbard. Area 1: including Bjørndalen, Coles Bay, Colesdalen and Grumant; Area 2: including Reindalen; Area 3: including Adventdalen, Flowerdalen, Janssonhaugen, Koslodalen and Nordenskiöld Land; Area 4: including Bjonehamna, Fredheim, Kapp Murdoch and Templet; Area 5: including Kapp Wijk and Area 6: including Austfjordnes. (C) Red foxes were sampled in Low-Arctic ecosystems of Varanger Peninsula and in the Sub-Arctic ecosystems in the mountain region around the lake Iešjávri, Finnmark County, Norway.

Fig. 2. Unrooted phylogenetic tree based on the complete genome nucleotide sequences of canine circovirus (CanineCV) obtained in this study and reference strains in the GenBank database (Table S2). The best-fit model of nucleotide substitution was determined using the Find Best DNA/Protein Model function implemented in MEGA X version 10.1.7. General Time Reversible (GTR) model with gamma distribution and invariable sites resulted optimal for the sequence data. The evolutionary distances were computed using the Maximum Likelihood method. Statistical support was provided by bootstrapping with 1000 replicates. Bootstrap values greater than 70% are indicated on the respective branches. The scale bars indicate the estimated numbers of nucleotide substitutions. Highlighted in black: Sequences generated in this study. In bold: Sequence of CanineCV 09-10F/2011 (MH454599) and CanineCV UCD3-478 (KC241983). On the left a traditional rectangular branch style of the tree. On the right a radiation branch style of the tree (black triangles: CanineCV identified in arctic foxes in this study, black circles: CanineCV identified in red foxes in this study, Empty circles: CanineCV reference sequences). Numbers in grey are the groups evidenced in this study and from 1 to 4 correspond to genotypes proposed by Niu et al. (2020).

Fig. 3. Unrooted phylogenetic tree constructed with the multiple gene approach: concatenated amino acid sequences of the replicase associated protein (Rep) and the capsid protein (Cap) of canine circovirus (CanineCV) generated in this study and reference sequences available from GenBank (Table S2). The best-fit model of nucleotide substitution was determined using the Find Best DNA/Protein Model function implemented in MEGA X version 10.1.7. Jones-Taylor-Thornton (JTT) model with gamma distribution and invariable sites resulted optimal for the sequence data. Phylogenetic tree was constructed using Maximum Likelihood method and bootstrap values were determined by 1000 replicates to assess the confidence level of each branch pattern. Bootstrap values greater than 70% are indicated on the respective branches. The scale bars indicate the estimated numbers of amino acid substitutions. Highlighted in black: Sequences generated in this study. In bold: Sequence of the CanineCV 09-10F/2011 (MH454599) and CanineCV UCD3-478 (KC241983). On the left a traditional rectangular branch style of the tree. On the right a radiation branch style of the tree (black triangles: CanineCV identified in arctic foxes in this study, black circles: CanineCV identified in red foxes in this study, Empty circles: CanineCV reference sequences). Numbers in grey are the groups evidenced in this study and from 1 to 4 correspond to genotypes proposed by Niu et al. (2020).